510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k100130

B. Purpose for Submission:

New device

C. Measurand:

Troponin I

D. Type of Test:

Quantitative, chemiluminescent enzyme immunoassay

E. Applicant:

Mitsubishi Chemical Medience Corporation

F. Proprietary and Established Names:

PATHFAST© cTnI-II test

PATHFAST cTnI Calibrators

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1215 Creatine Phosphokinase/creatine kinase or isoenzymes test system (troponin)

   21 CFR 862.1150 Calibrator secondary

2. Classification:

   Class II

3. Product code:

   MMI

   JIT

4. Panel:

   75 (Chemistry)

H. Intended Use:

1. Intended use(s):

   See indications for use statement below.

2. Indication(s) for use:
   PATHFAST cTnI-II is an in vitro diagnostic test for the quantitative measurement of cardiac Troponin I (cTnI) in heparinized or EDTA whole blood and plasma. Measurements of cardiac Troponin I are used to aid in the diagnosis of acute myocardial infarction. This method is for use in clinical laboratory or point of care (POC) settings.

   The PATHFAST cTnI Calibrators are for calibration for the PATHFAST system when used for the quantitative determination of cardiac Troponin I in human heparinized or EDTA whole blood and plasma.

3. Special conditions for use statement(s):

   For prescription use and point-of-care use

   Is not to be used for risk stratification

   In the labeling the sponsor states the following:

   - when using the 99th % cut-off, the PATHFAST cTNI-II test should be interpreted with at least 2 serial samples.
   - when using the ROC cut-off, the PATHFAST cTNI-II test should be interpreted with 3 serial samples.
   - when samples are collected in the early hours, it is not advisable to use the higher (ROC) cut-off.

4. Special instrument requirements:

   PATHFAST© Analyzer (k072189)

I. Device Description:

The PATHFAST© cTnI-II test is supplied in reagent kits. Each kit contains sufficient materials for 60 determinations. The calibrator materials are included with the reagent kit and are also available separately. Calibration kits and diluent kits are also provided separately.

Contents of the PATHFAST cTnI-II reagent kit

Component	Quantity
Reagent Cartridge	6 cartridges x 10 trays
Calibrator 1	2 vials
Calibrator 2	2 vials
Calibrator diluent	4 vials of 1.0 mL each

Reagent Cartridge: The reagent cartridge contains 16 wells. Wells 1, 6, 8, 9, 10, 12, 14, 15, 16 are empty. The other wells are filled with the following reagents:

Contents of the PATHFAST cTnI-II reagent cartridge

Reagent Description	Volume	Cartridge Well
Alkaline phosphatase (calf intestine) conjugated anti cTnI monoclonal antibody (mouse) in MES buffer (pH 6.0) with 0.007% zinc chloride, and 0.06% sodium azide as preservative	50 µl	2
Washing Buffer: Tris buffer (pH 7.5) with 0.05% sodium azide as preservative	400 µl	3, 4, 5
Magnetic particles coated with anti cTnI monoclonal antibody (mouse) in MOPS buffer	50 µl	7
Sample Dilution Buffer: Tris buffer (pH 8.2) with 0.05% sodium azide as preservative	25 µl	11
Chemiluminescent substrate: CDP-Star	100 µl	13

Calibrator 1: Lyophilized preparation containing MES pH 6.0, lactose, and enzyme free human serum, DTT
Calibrator 2: Lyophilized preparation containing cTnI complex, MES pH 6.0, lactose, and enzyme free human serum, DTT
Calibrator diluent: Aqueous solution with 0.05% sodium azide used for reconstituting Calibrators 1 and 2
Calibrator 1 and Calibrator 2 contain human serum obtained from donors who were confirmed negative for anti-HIV-1/2, HbsAg and Anti-HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Stratus CS Acute care Troponin I Testpak

2. Predicate K number(s):

   k033487

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Assist in the diagnosis of acute myocardial infarction. For use in clinical laboratory or point of care (POC) settings.	Same
Storage	2-8º C	2-8º C
Calibration Levels	6	6

Differences		
Item	Device	Predicate
Methodology	Chemiluminescent enzyme immunoassay	Solid phase radial partition immunoassay
Indications for use	Assist in the diagnosis of acute myocardial infarction. For use in clinical laboratory or point of care (POC) settings. Not for risk stratification	Assist in the diagnosis of acute myocardial infarction. For use in clinical laboratory or point of care (POC) settings. Risk stratification use
Sample Types	EDTA and Lithium heparin whole blood and plasma,	Heparinized Plasma
Reportable range	0.019 to 50 ng/mL	0 to 50 ng/mL

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition.*
CLSI EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures*: *A Statistical Approach; Approved Guideline.*
CLSI EP9-A2. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition.*
CLSI EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.*
CLSI C28-A2: *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition.*
ISO 17511:2003(E). *In vitro diagnostic medical devices—Measurement of quantities in biological samples—Metrological traceability of values assigned to calibrators and control materials*

ISO 14971:2000 *Medical devices - Application of risk management to medical devices*
ISO 13485:2003 *Medical devices - Quality management systems – Requirements for regulatory purposes*

L. Test Principle:

The PATHFAST cTnI-II test is a chemiluminescent enzyme immunoassay performed on the PATHFAST instrument.

Patient samples, whole blood or plasma, are dispensed by the operator into the designated area on the reagent cartridge. The instrument combines the patient sample, the antibody coated magnetic particles, and the alkaline phosphatase conjugate and incubates the mixture for 5 minutes at 37°C. During this incubation, the analyte in the patient sample binds to the antibody on the coated particles, and the alkaline phosphatase conjugate binds to the analyte-antibody coated-particle.

After the incubation, the instrument performs Bound/Free (B/F) separation using Magtration® technology to remove any excess unbound reagents. The chemiluminescent substrate is then added. The substrate is catalyzed by the bound alkaline phosphatase, which results in emission of photons.

The photo-multiplier tube in the PATHFAST instrument detects the photons that are emitted during the reaction. The chemiluminescent count is converted to analyte concentration values by the instrument based on the master calibration curve for the reagent lot.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Troponin (cTnI-II)

   Precision was determined with lithium (Li)-heparinized plasma and whole blood samples at four levels of the test. The study used 3 reagent lots on one instrument and one reagent lot on 3 instruments for a total of six different types of runs. Each sample was tested 20 times and the results are shown below. The results were calculated according to CLSI EP5-A2 guidelines.

   Whole blood precision

		Sample			
		LL	L	M	H
Across instruments/within reagent lot	Mean (ng/mL)	0.096	0.930	12.6	38.5
	SD	0.005	0.056	0.427	0.210
	% CV	4.7	6.0	3.4	0.5
Across reagent lots/within instrument	Mean (ng/mL)	0.096	1.01	12.4	41.3
	SD	0.007	0.092	0.241	2.64
	% CV	7.0	9.1	1.9	6.4

Lithium heparin plasma precision

		Sample			
		LL	L	M	H
Across instruments/within reagent lot	Mean (ng/mL)	0.146	0.924	11.6	30.4
	SD	0.006	0.062	0.762	2.447
	% CV	4.3	6.8	6.6	8.0
Across reagent lots/within instrument	Mean (ng/mL)	0.157	0.972	12.4	29.7
	SD	0.017	0.069	0.810	0.48
	% CV	10.8	7.1	6.5	1.6

In an additional study, inter-assay precision was assessed with Li-heparinized plasma samples at six levels within the test range, Four of the samples had values near the assay's two claimed cutoffs (0.264 ng/ml, and 0.029 ng/mL). Samples were tested over 20 days (n = 40 for each level tested) using one instrument and one lot of reagent. The results are shown in the table below.

Mean (ng/mL)	**SD**	**CV**
Troponin Conc. of 0.022		
Within-run precision	0.001	6.20%
Total precision	0.002	7.10%
Troponin Conc. of 0.029		
Within-run precision	0.001	5.10%
Total precision	0.002	6.10%
Troponin Conc. of 0.086		
Within-run precision	0.004	4.30%
Total precision	0.005	5.40%
Troponin Conc. of 0.251		
Within-run precision	0.009	3.70%
Total precision	0.01	3.90%
Troponin Conc. of 5.99		
Within-run precision	0.167	2.80%
Total precision	0.184	3.10%
Troponin Conc. of 25.9		
Within-run precision	0.823	3.20%
Total precision	0.954	3.70%

Point of care precision studies

Point of care precision studies were performed externally at three non-laboratory sites by physician assistants and medical office personnel to support the device's point of care claim.

Testing was performed at the three sites with 6 trained users (2 different operators for each POC site). Precision testing was conducted using 2 levels of control (n = 10 at each site) for 5 days per site. The following tables summarize the combined day to day and site to site results.

Troponin				
Level 1				
	Site 1	Site 2	Site 3	Overall
Mean ng/mL	0.267	0.247	0.255	0.256
SD	0.009	0.009	0.013	0.013
% CV	3.4	3.5	5.2	5.1
Level 2				
Mean ng/mL	7.81	7.37	7.91	7.70
SD	0.449	0.280	0.234	0.401
% CV	5.8	3.8	3.0	5.2

Additional precision studies were conducted at the point of care sites with lithium heparin whole blood samples. Ten samples were tested in duplicate at each site. Samples were tested within one day with one instrument at each site. All 3 sites used the same lot of reagent. In order to obtain a normal and abnormal level, some normal samples were spiked with control materials to obtain samples at elevated levels. The table below shows the results from all three sites.

Troponin results with whole blood samples (ng/mL)					
Site 1					
Sample	**Rep 1**	**Rep 2**	**Mean**	**SD**	**CV**
WB1	0.741	0.838	0.790	0.069	8.7%
WB2	2.39	2.32	2.36	0.049	2.1%
WB3	6.49	6.29	6.39	0.141	2.2%
WB4	4.39	4.24	4.32	0.106	2.5%
WB5	23.8	25.1	24.5	0.919	3.8%
WB6	0.153	0.146	0.150	0.005	3.3%
WB7	0.598	0.593	0.596	0.004	0.6%
WB8	0.057	0.059	0.058	0.001	2.4%
WB9	0.969	1.008	0.989	0.028	2.8%
WB10	0.075	0.079	0.077	0.003	3.7%
Site 2					
Sample	**Rep 1**	**Rep 2**	**Mean**	**SD**	**CV**

WB11	2.08	2.10	2.09	0.014	0.7%
WB12	10.0	10.1	10.1	0.071	0.7%
WB13	0.930	0.984	0.957	0.038	4.0%
WB14	0.103	0.105	0.104	0.001	1.4%
WB15	6.99	7.28	7.14	0.205	2.9%
WB16	1.31	1.29	1.30	0.014	1.1%
WB17	0.163	0.163	0.163	0.000	0.0%
WB18	0.032	0.034	0.033	0.001	4.3%
WB19	0.039	0.042	0.041	0.002	5.2%
WB20	0.032	0.033	0.033	0.001	2.2%
Site 3					
Sample	**Rep 1**	**Rep 2**	**Mean**	**SD**	**CV**
WB21	19.8	19.8	19.8	0.000	0.0%
WB22	0.309	0.306	0.308	0.002	0.7%
WB23	3.71	3.80	3.76	0.064	1.7%
WB24	30.0	31.0	30.5	0.707	2.3%
WB25	28.9	28.5	28.7	0.283	1.0%
WB26	0.055	0.052	0.054	0.002	4.0%
WB27	0.193	0.194	0.194	0.001	0.4%
WB28	0.041	0.042	0.042	0.001	1.7%
WB29	0.457	0.485	0.471	0.020	4.2%
WB30	0.159	0.166	0.163	0.005	3.0%

b. Linearity/assay reportable range:

Linearity studies were conducted on both whole blood and plasma samples. The linearity studies supported the sponsor's claimed assay range of 0.019 to 50 ng/mL.

For the whole blood study, lithium heparin whole blood samples with low and high values were diluted to produce 11 dilutions with values ranging from 0.013 to 50.1 ng/mL (n = 44). Slope = 1.01 (95 % CI = 1.00 – 1.02), intercept = 0.00 (95% CI = 0.00 – 0.00). The % recoveries for all samples ranged from 92.1 to 109.2 %.

For the plasma study, lithium heparin plasma samples with low and high values were diluted to produce 11 dilutions with values ranging from 0.0007 to 55.9 ng/mL (n = 44). Slope = 1.00 (95 % CI = 0.99 to 1.01), intercept = 0.00 (95% CI = 0.00 – 0.00). The % recoveries for all samples ranged from 92.4 to 103.1 %.

A hook effect study was conducted to assess samples above the range of the test. Samples were prepared by spiking troponin antigen purified from human

heart muscle into a buffer solution to 44,900 ng/mL. Serial dilutions were prepared with buffer solution. Theoretical concentrations of troponin in the sample were calculated from the initial concentration of the spiked sample and the dilution factor. The sponsor reports that samples between 50 ng/ml to 44,900 ng/mL return results above the range of the test.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

The cTnI-II assay is traceable to NIST standard SRM 2921. The primary calibrators are used to determine the concentration of the master calibrators that are prepared in house. The master calibrator's concentrations are verified using three PATHFAST instruments calibrated with the primary calibrators. The master calibrators are used to determine the concentration of the stock solutions for the working calibrators. The stock solution for the working calibrators is prepared in the same manner previously described. The working calibrators are prepared by diluting the stock solution to six specified levels of the test. Working calibrators are used to determine the concentration of the product calibrators. Concentrations are verified using three PATHFAST instruments calibrated with working calibrators. The product calibrators are prepared in lyophilized form at two levels for each test.

Stability

The sponsor conducted studies to determine the stability of the calibrators before reconstitution at 8º C. The stability studies support the sponsor's claimed stability of 12 months when stored refrigerated. The sponsor conducted open vial studies for the calibrators after reconstitution with the calibrator diluent. The studies supported the sponsor's claimed stability that reconstituted calibrators are stable for 3 days at 2-8 C and up to one month at -20º C or lower.

d. *Detection limit:*

LoB/LoD/LoQ studies were conducted using both lithium heparin plasma and whole blood samples. The study for whole blood utilized 8 PATHFAST instruments and one reagent lot. The LoB was determined by testing 60 replicates of a blank sample. The LoD was determined by testing 5 low samples in triplicate on 7 PATHFAST instruments. The LoQ was determined by identifying the lowest cTnI concentration of the LoD samples that showed less than 10 % CV. An identical study was performed using plasma samples. The statistics are presented in the table below.

Sample Type	LoB[a]	LoD[b]	LoQ[c]
Whole Blood	0.004 ng/mL	0.007 ng/mL	0.019 ng/mL
Plasma	0.003 ng/mL	0.005 ng/mL	0.015 ng/mL

[a] LoB = mean + 1.645*SD

[b] LoD = LoB + 1.645* pooled SD

[c] LoQ = minimum cTnI concentration with % CV < 10 %

The measuring range of the cTnI-II assay is from 0.019 ng/mL to 50 ng/mL.

e. Analytical specificity:

Samples with interfering endogenous substances at several levels in human Li-heparinized plasma samples were combined with plasma samples at 3 levels of troponin. Troponin levels were approximately 0.9 ng/mL, 5 ng/mL and 21 ng/mL. Samples were tested on the PATHFAST instrument. The measurement obtained was compared to the expected value, which is the value of the plasma samples with no interfering substances added. No significant interference as defined by recovery of +/- 10% was observed with bilirubin-conjugated and free (60 mg/dL), hemoglobin (1000 mg/dL), rheumatoid factor (500 IU/mL) and triglyceride (1000 mg/dL).

Cross reactivity was studied using potentially cross-reacting substances. The substances were added to a human normal Li-heparin plasma samples free from cTnI. Cross–reactivity was calculated as the apparent analyte concentrations divided by the working concentration of the cross-reactants. The results for the cross-reactivity studies are shown in the table below.

Cross-reactant	Working concentration	Cross reactivity
cTnT	1000 ng/mL	0.083%
cTnC	1000 ng/mL	0.000%
skTnI	250 ng/mL	0.093%

An extensive list of other compounds were evaluated for interference and were found to have no significant interference as defined by < 10% as summarized in the table below.

Drug	Highest level tested	cTnI concentration (ng/ml)	% recovery
Acetaminophen	20 mg/dL (1320 µmol/L)	1.35	102.0%
Acetylsalicylic	0.3 ng/mL (1.67 nmol/L)	1.37	103.5%

Acid			
Allopurinol	2.5 mg/dL (184 µmol/L)	1.32	99.5%
Ampicillin	5 mg/dL (143 µmol/L)	1.36	102.5%
Ascorbic Acid	3 mg/dL (170 µmol/L)	1.36	102.8%
Atenolol	1 mg/dL (37.6 µmol/L)	1.37	103.8%
Caffeine	10 mg/dL (515 µmol/L)	1.30	98.0%
Captopril	5 mg/dL (230 µmol/L)	1.36	102.8%
Digoxin	5 ng/mL (6.4 nmol/L)	1.26	95.2%
Dopamine	65 mg/dL (3.4 mmol/L)	1.32	100.0%
Erythromycin	20 mg/dL (273 µmol/L)	1.33	100.3%
Furosemide	2 mg/dL (61 µmol/L)	1.34	101.3%
Methyldopa	2.5 mg/dL (118 µmol/L)	1.34	101.5%
Niphedipine	6 mg/dL (173 µmol/L)	1.34	101.5%
Phenytoin	10 mg/dL (396 µmol/L)	1.31	99.2%
Theophylline	25 mg/dL (1390 µmol/L)	1.24	93.7%
Verapamil	16 mg/dL (0.33 µmol/L)	1.29	97.5%
No interferent added		1.32	100.0%

f. Assay cut-off:

This assay has 2 claimed clinical cutoffs. One cutoff (0.264 ng/mL) was determined in feasibility studies by ROC analysis and validated as described below. The second cutoff (0.029 ng/mL) is the 99th percentile of the reference interval for normal. See also Clinical cut-off section below.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed at three non-laboratory sites by physician assistants and medical office personnel.

Testing was performed at three sites with 6 trained users (2 different operators for each POC site). Method comparison testing was conducted with 57 lithium heparin plasma samples previously tested with the predicate method tested using the cTnI-II assay. Twenty samples were tested at each of the 3 sites. Samples with values outside of the measuring range were removed from the analysis of the data. The following table summarizes the Passing-Bablok regression results.

Sample range	Slope (ng/mL)	Confidence interval (ng/mL)	Intercept (ng/mL)	Confidence interval (ng/mL)	R
0.100 to 43.5	0.947	0.891-1.004	- 0.005	-0.025 to 0.019	0.994

b. Matrix comparison:

The sponsor conducted a matrix study to compare whole blood samples that were collected from patients in lithium heparin, sodium heparin, EDTA-Na and EDTA-K tubes. Plasma samples were prepared from each whole blood samples and were tested on the PATHFAST tests. Troponin values for the lithium heparin plasma samples (n=83) ranged from 0.020 to 46.3 ng/mL. 20 samples with values near the 99% cutoff and 10 samples with values near the ROC cutoff were included in the studies. Passing-Bablok regression analysis was performed with lithium heparin plasma as the reference matrix. The results in the tables below show acceptable correlation between lithium heparin plasma and other matrices.

Lithium heparin plasma compared to:	**N**	**Slope (95% CI)**	**Intercept (95% CI)**
Lithium heparin whole blood	83	0.97 (0.92 – 1.01)	0.003 (0.00 - 0.006)
Sodium heparin plasma	83	1.01 (0.99 – 1.03)	0.001 (0.002 – 0.004)
Sodium heparin whole blood	83	0.96 (0.92 – 0.99)	0.004 (0.000 – 0.010)
EDTA-K_2 plasma	83	0.99 (0.98 – 1.02)	0.00 (0.004 – 0.010)
EDTA- K_2 whole blood	83	1.00 (0.96 – 1.04)	0.003 (0.002 - 0.010)

3. Clinical studies:

a. Clinical Sensitivity:

A prospective clinical validation study was performed using patients presenting to the ER with a suspicion of AMI. The diagnosis of AMI was

performed according to ESC/ACC guidelines. Serial heparinized plasma specimens were collected from 333 patients within 12 hours of presentation to the ER. The blood sample collection times were at 0-2 hours, 2-6 hours and 6-12 hours of presentation to the ER. The group consisted of 72 patients with AMI and 261 without AMI. Clinical sensitivity and specificity were calculated based on both the ROC cutoff (0.264 ng/mL) and the 99th % cutoff (0.029 ng/mL) by time intervals after presentation to the ER. The results for the two cutoffs are presented in the tables below.

			#/TOTAL	95% CI
99th % Cutoff 0.029 ng/mL	0 to 2 h	Sens	73.6% (53/72)	(61.9% - 83.3%)
		Spec	92.7% (242/261)	(88.9% - 95.6%)
	2 to 6 h	Sens	93.1% (66/72)	(82.7% - 96.9%)
		Spec	93.1% (243/261)	(89.3% - 95.9%)
	6 to 12 h	Sens	91.7% (66/72)	(91.6% - 100%)
		Spec	91.6% (239/261)	(87.5% - 94.6%)

			#/TOTAL	95% CI*
ROC Cutoff 0.264 ng/mL	0 to 2 h	Sens	23.6% (17/72)	(14.4% - 35.1%)
		Spec	99.2% (259/261)	(97.3% - 99.9%)
	2 to 6 h	Sens	62.5% (45/72)	(50.3% - 73.6%)
		Spec	98.1% (256/261)	(95.6% - 99.4%)
	6 to 12 h	Sens	80.6% (58/72)	(69.5% - 88.9%)
		Spec	97.7% (255/261)	(95.1% - 99.2%)

The sponsor demonstrated through the matrix comparison studies that plasma and whole blood results are comparable matrices (see Matrix Comparison section 2 b above.

b. Clinical specificity:

See clinical sensitivity in 3 a. above.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

This assay has 2 claimed clinical cutoffs. One cutoff (0.264 ng/mL) was determined in feasibility studies by ROC analysis and validated as described below. The second cutoff (0.029 ng/mL) is the 99th percentile of the reference interval for normal.

The ROC cutoff was pre-established in a feasibility study consisting of 250 patients. The group consisted of 65 patients with acute myocardial infarction (AMI) and 185 patients without AMI. The value of 0.264 was selected as the appropriate cutoff value.

See section M5 below for additional information about how the 99th percentile reference interval cutoff was determined.

The sponsor presents the clinical performance for both the ROC cutoff and 99th% cutoff in the labeling. In addition, the sponsor states the following in the labeling:

- when using the 99th % cut-off, the PATHFAST cTNI-II test should be interpreted with at least 2 serial samples.
- when using the ROC cut-off, the PATHFAST cTNI-II test should be interpreted with 3 serial samples.
- when samples are collected in the early hours, it is not advisable to use the higher (ROC) cut-off.

5. Expected values/Reference range:

The sponsor conducted a reference range study to determine the 99th percentile at three sites within the US. Samples from 490 apparently healthy volunteers were collected and analyzed on the PATHFAST cTn-II assay. The sponsor's non-parametrical analysis represents the 99th percentile of the population tested. The upper 99th percentile of the reference interval was 0.029 ng/mL (95% confidence interval of 0.020 to 0.056).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.